

**EXPOSURE TO TRAFFIC-RELATED AIR POLLUTION AMONG PREGNANT WOMEN IN  
SOUTH TEXAS**

A Thesis

by

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## ABSTRACT

Traffic-related air pollution (TRAP) contributes significantly to overall ambient air pollution, and exposure to TRAP has been implicated in the development of an array of disease states, including asthma, chronic obstructive pulmonary disease (COPD), and lung cancer. TRAP exposure *in utero* and in early childhood can induce heritable epigenetic alterations. Additionally, *in utero* and early life TRAP exposure is linked to cognitive impairment, reduced lung function, asthma susceptibility, small for gestational age, and preterm birth. Furthermore, the risk of negative health outcomes increases with small incremental increases in particulate matter (PM) concentrations. Currently, many epidemiologic studies examining air pollution exposure in general or during pregnancy use sophisticated modeling to map exposure, or active air monitors for brief (24 to 48 hours) exposure assessments and use these findings to extrapolate study populations exposure across a lifetime or the course of pregnancy. Furthermore, the EPA utilizes stationary monitors, with limited coverage, to measure air quality and identify regions that fail to meet Air Quality Index (AQI) Standards. This study was conducted to assess the effect of microenvironment on TRAP exposure among pregnant women, a susceptible population, in a region with high childhood asthma incidence, Hidalgo County, Texas. Women were equipped with active air monitors and GPS devices for three separate 24-hour time points to examine the role the microenvironment has on PM exposure as well as examine variation between participants and between sampling

points. Participants' exposure was found to be highly variable and average PM<sub>2.5</sub> ranged from almost nonexistent, at 1.92µg/m<sup>3</sup>, to unhealthy, at 126.7µg/m<sup>3</sup>. Over 82% of participants experienced exposure outside of the "Good" range set by the EPA, and almost a quarter of participants experienced PM<sub>2.5</sub> exposure considered "Unhealthy" by the EPA. These results are intriguing given that these participants reside in a region that regularly falls into the "Good" AQI category. Moreover, for this population, the home microenvironment was the dominant microenvironment experienced and the largest single contributor to overall PM<sub>2.5</sub> exposure. This study speaks to the inherent variability associated with measuring PM in a population and the need for refined measures of exposure that capture microenvironment variation.

## **DEDICATION**

I would like to dedicate this thesis to my family and friends and loved ones who have been a source of love, support, and encouragement from before my scholastic career and continue to inspire me to follow my dreams.

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## NOMENCLATURE

1-AP	1-Aminopyrene
1-OHP	1-Hydroxypyrene
1-NP	1-Nitropyrene
B[a]P	Benzo[a]pyrene
$\mu\text{g}/\text{m}^3$	Micrograms per meter cubed
$\text{ng}/\text{m}^3$	Nanograms per meter cubed
OHNAAP	Hydroxy- <i>N</i> -acetyl-aminopyrene
OHNP	Hydroxy-nitropyrene
PAC	Polycyclic Aromatic Compound
PAH	Polycyclic Aromatic Hydrocarbon
$\text{pg}/\text{m}^3$	Picograms per meter cubed
$\text{PM}_{2.5}$	Particulate matter $>2.5\mu\text{m}$ in aerodynamic diameter
TRAP	Traffic-related air pollution

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## **1. INTRODUCTION**

### **Section 1.1 Air Pollution Overview**

Air pollution is a pervasive environmental health problem that contributes greatly to global morbidity and mortality. Broadly defined, air pollution is any form of contamination, physical, chemical or biological that alters the natural composition and properties of the atmosphere (WHO, 2014). Ambient air pollution is a complex chemical mixture produced by a wide range of activities and sources, both anthropogenic and natural, and includes compounds in the gaseous and particulate phases. The particulate phase consists of aerosolized solid and liquid phase compounds and represents the most hazardous constituent of ambient air pollution due to its association with several negative health effects (WHO, 2013). Particulate matter (PM) is classified by the aerodynamic diameter of the particle. Particles with a diameter between 10  $\mu\text{m}$  and 2.5  $\mu\text{m}$  are designated as coarse particulate matter or  $\text{PM}_{10}$ , and particles with a diameter less than 2.5  $\mu\text{m}$  are designated as  $\text{PM}_{2.5}$  or fine particulate matter.  $\text{PM}_{2.5}$  represents a significant health threat because its small size allows penetration deep into the lung into the gas-exchange region where the compounds associated with  $\text{PM}_{2.5}$  can readily cross into the blood stream (Pinkerton et al., 2000; WHO, 2013).  $\text{PM}_{2.5}$  exposure has been strongly linked to acute and chronic illnesses, including asthma, chronic obstructive pulmonary disease (COPD), lung cancer, and cardiovascular disease. Annually, over 3 million deaths are attributable to  $\text{PM}_{2.5}$  exposure (Lim et al., 2013; WHO, 2012; WHO,

2013). Additionally, PM<sub>2.5</sub> exposure during fetal development and early life has been correlated with decreased birth weight, preterm birth, delays in cognitive development, and insulin resistance (Hyder et al., 2014; Pereira, Belanger, Ebisu, & Bell, 2014; Thiering et al., 2013; Yorifuji, Kashima, Higa Diez, Kado, & Sanada, 2016).

### **Section 1.2 Traffic-related Air Pollution, PAHs and PACs**

PM<sub>2.5</sub> is composed of a heterogeneous mixture of many compounds adhered to a carbonaceous core; the composition can vary greatly based on location and source (Ji & Hershey, 2012; U.S. Environmental Protection Agency, 2004). Constituents include metals, ammonium, sulfate, nitrate, elemental carbon, and organic carbon. Organic carbon represents a major portion and contains a large class of incomplete combustion byproducts, namely polycyclic aromatic hydrocarbons (PAHs). PAHs are a group of over one hundred chemicals composed of fused benzene rings (containing carbon and hydrogen atoms), many of which are toxic and carcinogenic (WHO, 2013). These compounds are ubiquitous in the environment, resulting from power plant and other industry emissions, biomass smoke, cigarette smoke, and vehicular exhaust. Polycyclic aromatic compounds (PACs) include additional compounds formed during incomplete combustion reactions, or PAHs that have further reacted in the atmosphere, such as nitrated or oxygenated PAHs. These PACs have been suggested to possess increased mutagenicity and toxicity and may be produced at higher concentrations in vehicle exhaust (Schuetzle, Riley, Prater, Harvey, & Hunt, 1982). In the U.S., the greatest single

contributor to ambient particulate matter pollution is traffic-related emissions, with diesel engine exhaust contributing a significant proportion (Hodan & Barnard, 2004; Woghiren-Akinnifesi, 2013). Therefore, traffic-related air pollution (TRAP) represents a significant source of pollution, particularly for susceptible groups like children, asthmatics, pregnant women, and immunosuppressed individuals.

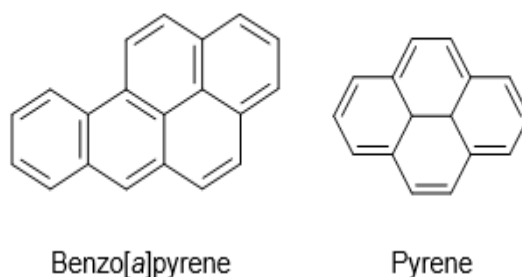
Despite the vast amount of epidemiological data that indicates exposure to PM<sub>2.5</sub> and PAHs *in utero* or during early childhood increases the risk of negative health outcomes, such as low birth weight, respiratory infection, asthma, and allergic disease, there are still major gaps in our understanding of interplay between PM<sub>2.5</sub> and PAH/PAC exposure and health effects later in life (Bowatte et al., 2015; Hyder et al., 2014; Jedrychowski et al., 2013; Perera et al., 2009). Furthermore, as traffic emissions represent a prominent source of PAHs and PACs, the health effects of traffic-related air pollution (TRAP), need to be explicitly explored, especially in light of growing evidence that implicates exposure to TRAP *in utero* as a risk factor for asthma later in life (Clark et al., 2010; Nishimura et al., 2013; Zhou et al., 2013).

In order to delineate the effects of TRAP exposure on health outcomes, TRAP should be quantified in a selective and sensitive manner. Furthermore, efforts to quantify exposure to TRAP should be robust enough to capture microenvironment variation, as macro-level estimations utilizing regression models and stationary monitors may not be sensitive enough to identify the variation that occurs throughout a day, as individuals move from their residence, to their schools, places of work and other sites of variable

exposure. In order to conduct robust risk assessments for health effects of TRAP exposure, biomarkers of exposure are of critical need. The heterogeneous nature of TRAP complicates the ability to accurately measure exposure, as TRAP is a diverse and fluctuating mixture of hundreds to thousands of compounds. Thus, rather than attempt to measure this vast array of compounds, a surrogate or series of surrogates have been used as proxies for overall TRAP exposure. Several commonly used markers of TRAP include elemental carbon, total carbon, and overall PM<sub>2.5</sub> concentration. Measurements of PM<sub>2.5</sub> are convenient because they can be measured in real time and offer a metric that can be readily compared. Several studies have established a dose dependent association with PM<sub>2.5</sub> and negative outcomes, with incremental increases of 10µg/m<sup>3</sup> of PM<sub>2.5</sub> associated with significantly increased risk of myocardial infarction, asthma symptoms, and overall mortality, as well as small for gestational age and low birth weight when exposed during pregnancy (McCormack et al., 2009; Shi et al., 2016; Stieb et al., 2016; WHO, 2013). Despite the utility of PM<sub>2.5</sub> measurements, these metrics alone are not effective as a biomarker because PM<sub>2.5</sub> cannot be reliably calculated into a relevant dose of PAHs. Furthermore, due to the variable nature of PM<sub>2.5</sub>, exposure to the same concentration of PM but from different sources is likely to result in exposure to different compounds and perhaps differential toxicity.

The measurement of PAHs is one method to look at dose of exposure, and several PAHs have been classically used as proxies for the measurement of TRAP. Two of the

most regularly used PAH proxies are pyrene and benzo[a]pyrene (B[a]P) (Figure 1.1), which are PAHs present in high concentrations formed during incomplete combustion.



**Figure 1.1 Chemical structures of representative PAHs.** Benzo[a]pyrene and pyrene have been used extensively as proxies for overall PAH exposure.

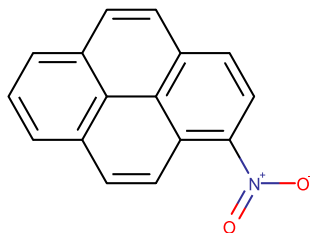
Pyrene is ubiquitous in organic combustion emissions and generally occurs in high concentrations allowing for ease of detection. B[a]P is often used as a proxy for overall PAH carcinogenicity because it is a known human carcinogen (Jung et al., 2010; Perera, Tang, Whyatt, Lederman, & Jedrychowski, 2005). Furthermore, the exposure pathways, urinary metabolites of B[a]P and pyrene and half-lives are well characterized, allowing for researchers to measure concentrations in the environment, as well as at the individual level through metabolites in biological samples (Morgott, 2014). The urinary metabolite 1- hydroxypyrene (1-OHP) has been extensively characterized and validated as a biomarker of PAH exposure in many human populations and is widely accepted due to the presence of pyrene in most PAH mixtures (Morgott, 2014; Scheepers et al., 2004).

However, the use of 1-OHP, as well as B[a]P metabolites, to classify overall PAH exposure is not without limitation. Both pyrene and B[a]P are pervasive in the emissions of other organic fuels. This can pose a significant barrier to measuring TRAP in the presence of other PAH sources such as industrial emissions, cigarette smoke, and fires. Additionally, the metabolites of these compounds may fail to correlate with overall exposure or may be masked by other behaviors that contribute to PAH exposure, such as environmental tobacco exposure or the consumption of foods prepared by grilling, roasting, or broiling (Morgott, 2014). In fact, several researchers have called for more specific biomarkers of exposure to TRAP. Hara et al (1997) examined exposure to TRAP among city sanitation workers via personal air monitors and compared total diesel exhaust exposure to urinary 1-OHP. Strikingly, the researchers did not identify a relationship between diesel exhaust exposure and 1-OHP and noted that 1-OHP may be an imperfect biomarker when attempting to quantify low level exposures, which are frequently encountered in ambient atmosphere (Hara, Hanaoka, Yamano, & Itani, 1997). Scheepers et al (2004) examined urinary 1-OHP in a population of shale miners and identified no significant difference in metabolite concentration between underground workers exposed to high levels of diesel exhaust versus aboveground workers (Scheepers et al., 2004). These results were surprising as the underground workers were exposed to levels of diesel exhaust at an order of magnitude greater than surface workers. Although other studies have effectively utilized 1-OHP as a marker of air pollution, the utility of 1-OHP as a specific biomarker marker of TRAP is limited, especially in the presence of other PAH

sources. To better examine exposure specific to TRAP, a biomarker must be used that correlates well with sources of TRAP and not simply general combustion (Morgott, 2014).

### Section 1.3 1-Nitropyrene

One proposed biomarker of TRAP is 1-nitropyrene (1-NP) and its associated metabolites. 1-NP is technically classified as a PAC and belongs to a class of compounds called nitrated polycyclic aromatic hydrocarbons (NPAHs). 1-NP is produced during incomplete combustion in the presence of atmospheric nitrogen and is composed of four peri-fused benzene rings with a nitro functional group (Figure 1.2).



**Figure 1.2 1- Nitropyrene structure.**

1-NP has been identified as genotoxic and mutagenic in several *in vivo* and *in vitro* models and is classified as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC), “a substance possibly carcinogenic to humans” (IARC, 1989; National Toxicology Report, 1996; Salmeen, Durisin, Prater, Riley, & Schuetzle, 1982; Schuetzle et al., 1982). It also contributes greatly to the overall carcinogenicity of diesel



engine exhaust, a substance classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen, “a substance carcinogenic to humans” (IARC, 2012).

1-NP is the most common NPAH present in diesel engine exhaust and a very common NPAH in ambient atmosphere (Bamford, Bezabeh, Schantz, Wise, & Baker, 2003; Rosenkranz, 1982). The amount of 1-NP found in the atmosphere can vary greatly and is dependent on traffic volume, temperature, season, and time of day. Ambient air in regions with dense traffic can contain 1-NP near or above 1 ng/m<sup>3</sup>; however, urban and suburban levels are usually measured in hundreds or tens of pg/m<sup>3</sup>. The lowest recorded levels are found in extremely remote regions and at levels in the low pg/m<sup>3</sup> range (IARC, 2013). Seasonal peaks occur during winter months with daily peaks coinciding with periods of increased traffic volume. The seasonal peaks are attributed to decreased sunlight, which in turn decreases the occurrence of photolytic degradation. Additionally, other variables can influence the concentration of 1-NP emitted by diesel engines, including engine efficiency, engine workload, fuel type, fuel age, and the presence of emissions controls (Schuetzle & Perez, 1983).

1-NP has also been identified in the emissions of propane and liquefied petroleum gas, crankcase oils, and certain grilled or fried foods. Unlike other biomarkers of combustion that can originate as both primary emissions or form during secondary atmospheric reactions, 1-NP is not formed through the atmospheric reaction of pyrene with hydroxyl or NO<sub>x</sub> radicals (2- and 4-nitropyrene are the species formed instead) (Pitts

et al., 1978). Furthermore, 1-NP has not been identified in the mainstream or side stream smoke of cigarettes, a characteristic that expands the utility of this compound as a biomarker of TRAP (IARC, 2013). Thus, 1-NP has the potential to serve as a valuable biomarker of exposure to TRAP as the combustion of diesel fuel remains the foremost source of atmospheric 1-NP and skewing due to other sources like secondary atmospheric formation, environmental tobacco smoke, and fires is unlikely (Atkinson & Arey, 1994; IARC, 2013; Kielhorn, Wahnschaffe, Mangelsdorf, & International Programme on Chemical Safety, 2003; Wang et al., 2015).

#### **Section 1.4 1-Nitropyrene Metabolism**

The metabolism of 1-NP has been characterized in a variety of animal and cell models and major metabolites have been identified in rodents and humans (IARC, 2013; National Toxicology Report, 1996). Despite significant scientific investigation into 1-NP metabolism in rodent models, the current understanding of human 1-NP metabolism is limited. This is in part due to the fact that human exposure to 1-NP occurs concurrently with many other compounds in a diesel engine exhaust mixture. Nonetheless, the general schema of metabolism is understood. For the majority of the population it is expected that inhalation is the major route of exposure, with dermal exposure and ingestion also contributing to a lesser extent depending on personal behaviors. Upon inhalation, most 1-NP is rapidly cleared by the upper respiratory tract and absorbed into the bloodstream within an hour (National Toxicology Report, 1996). Particle bound 1-NP

may also be caught in the upper respiratory system and then ingested via mucociliary clearance (J. D. Sun, Wolff, Aberman, & McClellan, 1983). From there, 1-NP enters the gastrointestinal tract and is rapidly absorbed into the blood stream (Van Bakkum, Van den Broek, Scheepers, Noordhoek, & Bos, 1999). Once in the bloodstream 1-NP is transported to the liver and kidneys and metabolized, with high levels of macromolecule binding occurring in both the kidneys and liver.

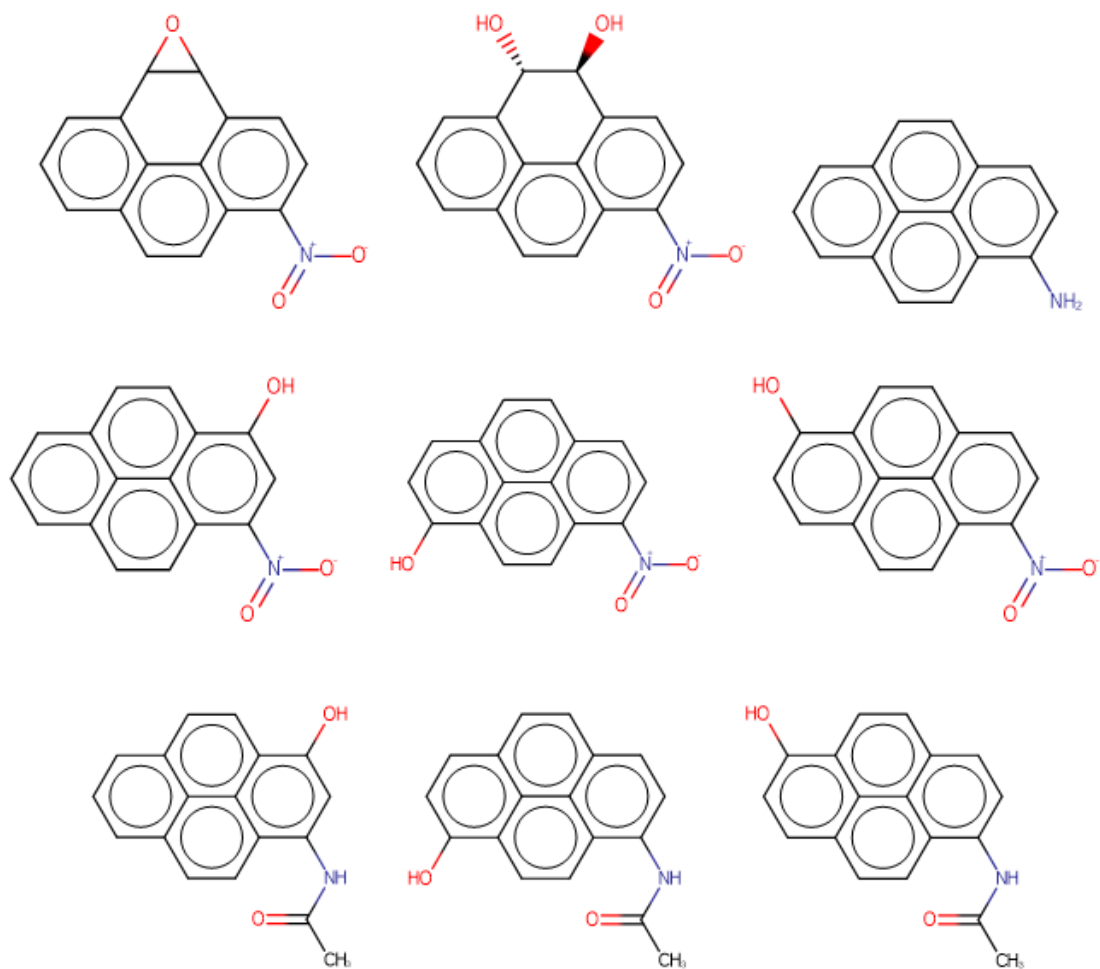
Phase I metabolism occurs in one of two ways, nitroreduction or oxidation. The metabolic pathway undertaken is determined by available oxygen, with anaerobic conditions leading to nitroreduction and aerobic conditions favoring oxidation (El-Bayoumy & Hecht, 1983). Additionally, the metabolic pathways are not exclusive, as a considerable portion of 1-NP metabolites are oxidized and then reduced. Nonetheless, both of these pathways are biologically significant, and the metabolites formed via nitroreduction and oxidation can react with DNA forming adducts (Howard, Flammang, & Beland, 1985). Ring oxidation, which occurs under aerobic conditions, produces a range of hydroxy-1-nitropyrenes as well as K region nitropyrene epoxides (National Toxicology Report, 1996). These epoxides are deleterious as they are extremely unstable due to their high ring strain and are capable of covalently bonding with proteins as well as DNA; additionally these epoxides can also be further oxidized to form dihydrodiols (National Toxicology Report, 1996). Cytochrome P-450 (CYP) 3A3 and 3A4 specifically, were identified as the enzymes responsible for 1-NP metabolism in human microsomal samples as they were the only class of CYPs that exhibited catalytic activity towards 1-NP

(Silvers et al., 1992). However, other CYP enzymes may be involved in 1-NP metabolism as inhibition of CYP3A3 and 3A4 reduced 1-NP metabolism to 30-40%, but did not prevent it completely. Additional studies using human breast cells, immortalized breast cells, and immortalized breast epithelial cells have identified CYP 1A1 and 1B1 as enzymes capable of mononitropyrene metabolism with the potential of forming reactive metabolites capable of forming macromolecule adducts (Sun, Yuan-Wan Guengerich, F P Sharma, Arun Boyiri, Telih Amin, Shantu el Bayoumy, Karam, 2004).

The other main phase I metabolic pathway of 1-NP, nitroreduction, has been observed *in vivo* and *in vitro* in a variety of animal systems and can be catalyzed by both mammalian enzymes such as xanthine oxidase, aldehyde oxidase, or DT-diaphorase as well as bacterial nitroreductases. The reduction of 1-NP is sequential and the metabolites formed in the process exhibit a broad range of properties. Metabolites such as 1-aminopyrene (1-AP) are less toxic than 1-NP and do not appear to be mutagenic. Furthermore, 1-AP can be readily conjugated by phase II enzymes allowing for elimination (Manning, Cerniglia, & Federle, 1986). However, intermediates formed during the reduction of 1-NP to 1-AP, such as N-hydroxy-1-aminopyrene and 1-nitrosopyrene can form adducts with DNA and proteins (King, Kohan, George, Lewtas, & Claxton, 1990). Research has demonstrated that both bacterial and mammalian cell lines can form DNA adducts during the nitroreduction of 1-NP (Edwards, Batmanghelich, Edwards, Parry, & Smith, 1986). Additionally, human umbilical cord vein endothelia exposed to low levels of 1-NP display DNA damage and increased production of reactive

oxygen species (ROS). However, when nitroreductase activity is inhibited, levels of DNA damage and ROS significantly decrease (Andersson, Piras, Demma, Hellman, & Brittebo, 2009). These metabolic products of phase I enzymes are then metabolized by phase II enzymes, responsible for conjugating the newly oxidized or reduced compounds with glutathione, glucuronic acid, or sulfuryl groups forming metabolites that are readily excreted into the urine or into bile and then feces. (Jancova, Anzenbacher, & Anzenbacherova, 2010; Medinsky, Shelton, Shelton, & McClellan, 1985). However, a portion of 1-NP is reabsorbed, facilitated by intestinal bacteria (Medinsky et al., 1985). Enteric bacteria are believed to cleave the phase II conjugates allowing for secondary metabolism to occur (Kataoka, Kinouchi, Akimoto, & Ohnishi, 1995). Wild type mice administered 1-nitropyrene epoxides, already conjugated to glutathione, form DNA adducts whereas antibiotic treated mice do not (Kinouchi, Kataoka, Miyanishi, Akimoto, & Ohnishi, 1993). Furthermore *in vitro* studies have shown a variety of bacterial  $\beta$ -lyases are capable of transforming 1-nitropyrene epoxide conjugates into mutagenic and biologically active compounds (Kataoka et al., 1995). The compounds are then recirculated and can be nitroreduced through human or bacterial enzymatic processes. In addition to contributing to 1-NP secondary uptake, enteric bacteria are believed to have a substantial role in the metabolism of 1-NP in mammals. Human, mouse and rat intestinal flora have all been shown to reduce 1-NP to 1-AP, as well as other minor nitroreduction products (King et al., 1990). Furthermore, the formation of 1-NP metabolites, primarily 1-AP, is greatly reduced in antibiotic-treated or germfree animals.

In one study, in which germ free and conventional rats were treated with 1-NP via oral gavage, no detectable amount of 1-AP was identified in the feces of germ free rats, however 1-AP was found in the feces of conventional rats (El-Bayoumy, Sharma, Louis, Reddy, & Hecht, 1983). A separate study compared the formation of 1-NP metabolites and metabolite binding in the lung and liver of antibiotic treated rats and conventional rats. Antibiotic treated rats displayed significantly lower metabolite binding in the lung when compared to control animals. Additionally, control animals displayed greater amounts of 1-AP and 1-acetylaminopyrene than the treatment group, illustrating the potential role enteric bacteria play in 1-NP reduction (Ayres, Sun, & Bond, 1985). Furthermore, human fecal bacteria treated with 1-NP are capable of reducing the compound to 1-AP, an indication that unaltered 1-NP in feces can still be reduced within the lower gastrointestinal tract. These studies point to the intricate relationship that mammalian microbiota is believed to play in 1-NP metabolism and the likely variation expected due to the complexity and variation seen in human microbiota (Mentis, Gypas, & Mentis, 2013).



**Figure 1.3 Biologically significant metabolites of 1-nitropyrene.**

(From left to right): Top: 1-nitropyrene-4,5-oxide, 1-nitropyrene-trans-4,5-dihydrodiol, 1-aminopyrene. Middle row: 3-hydroxy-1-nitropyrene, 6-hydroxy-1-nitropyrene, and 8-hydroxy-1-nitropyrene. Bottom row: 3-hydroxy-*N*-acetyl-1-aminopyrene, 6-hydroxy-*N*-acetyl-1-aminopyrene, and 8-hydroxy-*N*-acetyl-1-aminopyrene.

The assessment of 1-nitropyrene metabolites in humans is a recent development and has been limited to a handful of studies. Toriba et al (2007) was the first to examine a range of 1-NP metabolites in human urine and identified 3-, 6-, and 8-hydroxy-1-nitropyrene (3-, 6-, and 8-OHNP) and 3-, 6-, and 8-hydroxy-*N*-acetyl-1-aminopyrene (3-,

6-, and 8-OHNAAP) as the major metabolites (Figure 1.3). Researchers reported that unlike in animal models, OHNPs were the predominant human metabolites and that the 6- and 8- position OHNPs and OHNAAPs were preferentially formed over the 3- position metabolites (Toriba et al., 2007a).

Miller-Schulze et al. (2013) identified three metabolites (6-, 8-OHNP, and 8-OHNAAP) consistently in an exploratory study among Chinese taxi drivers, however the authors did not identify a significant increase in metabolite concentration after elevated occupational 1-NP exposure (Miller-Schulze et al., 2013). The sampling time points used may have complicated the relationship between exposure and metabolite elimination. The authors estimated that the half-lives of the OHNP and OHNAAP metabolites are roughly 10 to 12 hours. As such, their urine sampling times may have missed the peak excretory point. Laumbach et al. (2009) examined urinary concentrations of 1-AP in spot urine samples from human volunteers who were exposed to 60 minutes of clean filtered air and 60 minutes of diluted diesel exhaust in a controlled exposure chamber at least a week apart. In this study, the majority of subjects had higher 1-AP levels in their urine following exposure to diesel exhaust when compared to clean filtered air. However, significant variation between subjects was observed which made it difficult for the authors to draw major conclusions from the results. A secondary analysis identified variation in the rate of 1-AP excretion between subjects, with one group reaching peak excretory levels at roughly 5.5 hours after exposure and the other group believed to have a peak excretory value beyond the 24 hour period of the study (Huyck et al., 2010).



Galaviz (2013) examined 1-nitropyrene exposure and two 1-NP urinary metabolites (8-OHNP and 8-OHNAAP) among individuals who regularly commuted across the California-Mexico border as well as those who did not commute across but lived proximal to the border. Border commuters, the high exposure group, were found to be significantly exposed to more PM<sub>2.5</sub>, and 8 times more 1-NP than those who did not commute across the border. Furthermore, the urinary concentration of 8-OHNP and 8-OHNAAP were two and three times higher, respectively, than their non-commuting counterparts (Galaviz, 2013). This research in this thesis draws upon the growing body of human 1-NP exposure assessment with the goal of exploring the use of 1-NP as a biomarker of TRAP in pregnant women residing in Hidalgo County.

### **Section 1.5 Study Rationale and Aims**

Air pollution represents a severe and growing environmental problem along the U.S.-Mexico Border, as this dynamic region has experienced rapid population growth and development in the past 30 years. The U.S. border population has increased by 5 million from 1980 to 2005 and is expected to double within 30 years, as economic conditions have favored increased industrial expansion and trade between the U.S. and Mexico. This increase in industry, population, and trade has led to an increase in many environmental issues, including air pollution (Lee et al., 2013). Emissions of fine particulate matter in Hidalgo County, TX (our study location) have continuously risen. Childhood asthma is an emerging as a priority public health problem in Hidalgo County where rates are significantly higher than the overall state rate (11.4% versus 7.4%) and hospitalization

rates are 50.5% higher than the state average. The link between air pollution exposure during critical developmental windows, namely, prenatal or early infancy periods, and the development of childhood asthma has become increasingly accepted, however has yet to be explored as a potential etiologic factor in this region. Moreover, the lack of validated individual exposure biomarkers has limited knowledge of PAHs/PACs on asthma risk following prenatal air pollution exposure.

Therefore, the primary research objective of this project was to characterize prenatal exposure to traffic-related air pollution in women living in Hidalgo County, where the rates of childhood asthma are some of the highest in the state, and investigate the potential for 1-NP metabolites to serve as biomarkers of exposure to TRAP. The focus of this thesis is on the characterization of prenatal exposure through the following specific aims:

Aim 1. Our first aim was to utilize traditional active air sampling techniques to assess personal PM<sub>2.5</sub> exposure in women recruited in their third trimester of pregnancy from prenatal clinics in the McAllen-Edinburg urban area.

Aim 2. The second aim was to investigate the contribution of various microenvironments to overall PM<sub>2.5</sub> exposure.

Future work in this population will focus on measuring individual PAH and NPAH urinary metabolites, 1-OHP, 3-,6-, and 8-OHNP, and 3-,6-, and 8-OHNAAP, and compare levels with personal PM<sub>2.5</sub> exposure metrics.

## **2. METHODS**

### **Section 2.1 Population**

Hidalgo County is located in the Southern tip of Texas, along the U.S.-Mexico border and close to the Gulf of Mexico. Hidalgo County contains the McAllen-Edinburg-Mission Metropolitan Statistical Area, and is home to over 842,000 residents. Hidalgo County is also a very young region, with roughly 33% of residents under the age of 18. Additionally, the region experienced an 8.7% population increase from 2010 to 2015, over twice as fast as the national average (US Census Bureau, 2015). At over 90% percent, the vast majority of residents are Hispanic or Latino and 85% of residents speak a language other than English at home (US Census Bureau, 2015). The major economic activities are centered on agribusiness, agriculture, food processing, tourism, and petroleum (Garza, 2010). The county is also considerably more impoverished than the national or state average, as the median household income for the county was \$34,952 in 2014, and roughly 33% of the population is in poverty.

### **Section 2.2 Study Participants**

Participants (n = 18) were recruited in local prenatal health clinics by medical assistants and community health workers. Eligibility criteria required that participants be: 21 to 35 years of age, nonsmokers residing with nonsmokers, in their third trimester of pregnancy at time of sampling, current residents of Hidalgo County, and planning to deliver at the

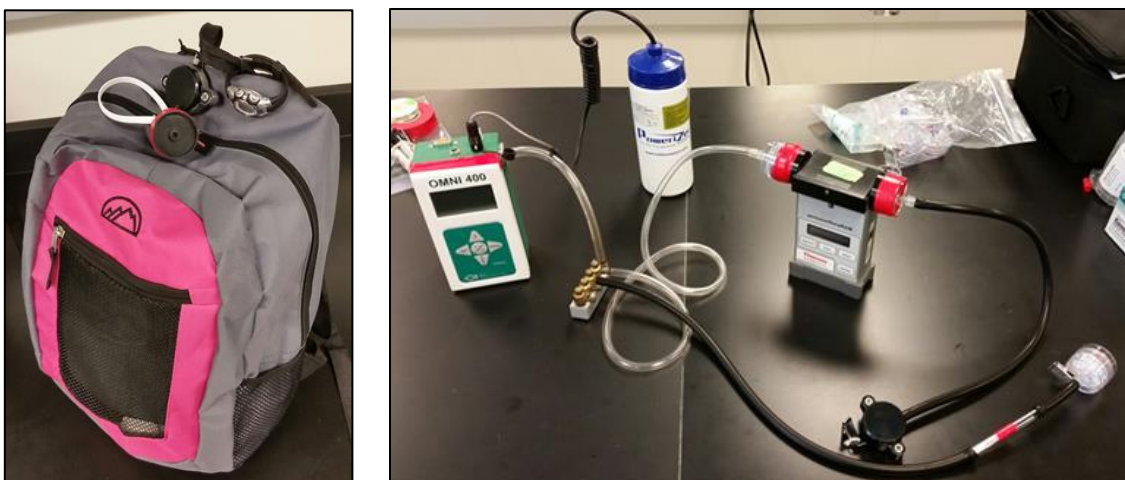
Rio Grande Regional Hospital in McAllen. Potential candidates for participation were screened by clinic staff at the regional clinics where they were receiving prenatal care. Viable candidates were introduced to the study by clinic staff, and if they expressed interest local community health workers contacted them to further explain the study process and provide informed consent (IRB2014-0813F).

### **Section 2.3 Overall Study Design**

The study design involved repeated measures consisting of three separate sampling points occurring at 2-week intervals. At the time of consent or at the first sampling time point each participant was administered a questionnaire detailing personal behaviors, home and work environment, and lifestyle factors related to traffic exposure. At each sampling time point, the participants were provided with air sampling equipment in a small backpack by a community health worker and instructed to wear the backpack or keep the backpack in their breathing zone for 24 hours. This backpack contained an active air monitor, as well as a GPS device, and a humidity and temperature logger. The following day the participant's first morning urine void was collected along with the backpack and returned to the community health worker. The third (and final) sampling point included the collection of a hair sample and a venous blood draw at a prenatal wellness clinic. At the end of each 24-hour sampling period, a 24-hour questionnaire was administered to the participants, which detailed the activities conducted in the past day.

## **Section 2.4 Air Pollution Exposure Assessment**

At two-week intervals participants were delivered a portable air monitor in a small backpack and asked to wear it or keep it near breathing level for 24 hours. After 24 hours a community health worker collected the air monitor and delivered it to the field laboratory. The air sampling equipment contained in the backpack (Figure 2.1) consists of an active nephelometer (personal data ram pDr 1000, Thermo Scientific) that detected PM<sub>2.5</sub> in real time and logged measurements at 10 second intervals. In order to size select for PM<sub>2.5</sub> the pDr's inlet was equipped with a 2.5 µm impactor connected to a sampling pump (Omni-400 personal sampling pump, BGI by Mesa Labs) operated at a continuous flow rate of 4L/minute with the PM<sub>2.5</sub> collected on 37mm pre-weighed Teflon filters. A second line from the BGI pump was split to draw air at 1L/minute through a 37mm PTFE filter (Zefluor™ Supported PTFE, Pall Corporation #28139-222) for PAH and 1-NP analysis, followed in line by a XAD resin tube (XAD-2 p-asinidine, SKC Inc. # 226-30-07) for volatile PAH analysis. Sampling pumps and inlets were calibrated before and after each 24 hour sampling period and averaged after each run. After the end of each 24 hour sampling period the Teflon filter, PTFE filter, and XAD tube were removed from the monitor, placed in individual plastic petri dishes, bagged and stored at -20°C. The PM<sub>2.5</sub> data was then downloaded immediately after each sampling period along with temperature and humidity (HOBO data logger, UX100-003) and GPS data (Qstarz International, BT-Q1000XT).



**Figure 2.1 Sampling backpack and contents.** Sampling inlets for PTFE filter (with red wrap) and for Teflon filter are visible outside of the backpack. The pDr and pump are contained within the backpack.

The Teflon filters were weighed on a microbalance with 1- $\mu$ g resolution in a temperature and humidity controlled room. Total mass of PM<sub>2.5</sub> was calculated by comparison with the original filter mass.

## **Section 2.5 Urine Collection & PAH/PAC Metabolite Analysis**

Participants were provided with a sterile 120 ml plastic urine container (BD Vacutainer Urine Collection Cup, #364975) and instructed to collect their first morning void upon waking the next day. Participants were then instructed to keep urine cool until transport on ice to the field laboratory by a community health worker. Upon receipt of urine, the volume was measured and recorded. Next, deionized water was added until the final volume reached 100 ml. The urine was then partitioned into a 5ml aliquot for creatinine analysis (BaylorScott and White Healthcare), with the remainder stored in polypropylene

50 ml Falcon tubes. All urine was stored at -20°C at the field laboratory until transported on dry ice and stored at -80°C until sample analysis in our laboratory in College Station.

Urine was thawed and 5 ml 4M sodium acetate buffer was added, followed by 75µl 1M HCl to adjust to pH 5. Deuterated internal standards, were synthesized, as described below, according to Toriba et al (2007), Rosser et al (1996) and Abe, Y., & Saito, M. (1965), and spiked into the urine and incubated with 75 µL β-glucuronidase/aryl sulfatase at 37°C for 4 hours in a shaking water bath in order to deconjugate the PAC metabolites. Upon deconjugation, the urine was incubated with 100g blue rayon for 1 hour at room temperature while in a orbital shaker at 60 rpm. The blue rayon was then transferred to a funnel and rinsed with deionized water and then dried in an empty 20 cc Sep-Pak cartridge. The dried blue rayon was then sonicated in 20 mL methanol: ammonium (50:1) for 30 minutes while protected from light. This solution and the subsequent methanol rinses of the blue rayon were filtered and blown down under nitrogen to dryness using a TurboVap. The extract was then dissolved in 5 mL ethyl acetate: methanol; (1:1), vortexed and then eluted through a Sep-Pak Alumina A cartridge. The extract was collected and reduced to dryness under nitrogen and then suspended in 300 µl methanol until analysis using LC-MS/MS.

## Section 2.6 Metabolite Standard Synthesis

Briefly, 5.002 g pyrene was acetoxylation via reflux with 11.027 g lead (IV) acetate in 30 ml 9:1 toluene: acetic acid for 6 hours at 80°C. The resulting product was separated via column chromatography using 10% ethyl acetate: hexane as the elutant. The acetoxypyrene eluted as the second bright blue band when visualized with long wave UV light. This was collected and dried under nitrogen. The acetoxypyrene was then nitrated following the procedure outlined by Abe, Y., & Saito, M. (1965) with minor revisions, briefly 1 g acetoxypyrene was dissolved in 8ml glacial acetic acid while lightly stirring at 45°C and then nitrated with 0.9 ml concentrated nitric acid. The solution turned dark red and acetoxynitropyrene precipitated out as bright yellow precipitate. The acetoxynitropyrene was filtered, dried under nitrogen. To deprotect the acetoxynitropyrene, the compounds were dissolved in 4% sodium hydroxide. When the solution turned deep purple the liquid was filtered and then concentrated hydrochloric acid was added dropwise until the solution turned acidic, as monitored by pH paper. Upon acidification, the solution turned bright red and then clear as red hydroxynitropyrene precipitated out. The precipitated was filtered, washed, and dried under nitrogen. Hydroxy-*N*-acetyl-1-aminopyrene utilized the hydroxypyrenes as the precursor. These were dissolved in methanol, with Raney Copper and sodium borohydride, stirred at 40°C and then filtered into acetic anhydride. Nitropyrene was synthesized following the nitration protocol from Abe, Y., & Saito, M. (1965), however using pyrene as the precursor instead of acetoxypyrene. Hydroxypyrene utilized the first



step of hydroxynitropyrene synthesis, however skipped the nitration step and proceeded directly to deprotection. Deuterated standards were synthesized following the same protocols, using deuterated d<sub>10</sub>-pyrene as the precursor.

### 3. RESULTS

#### Section 3.1 Summary

A total of 17 participants were sampled, resulting in 50 independent sampling events (one participant was unable to complete the third sampling run). Average exposure to PM<sub>2.5</sub> was quite variable between participants and between sampling time points, as seen in Table 3.1 and Figure 3.1. Furthermore, participants' exposures were quite variable between home and commute.

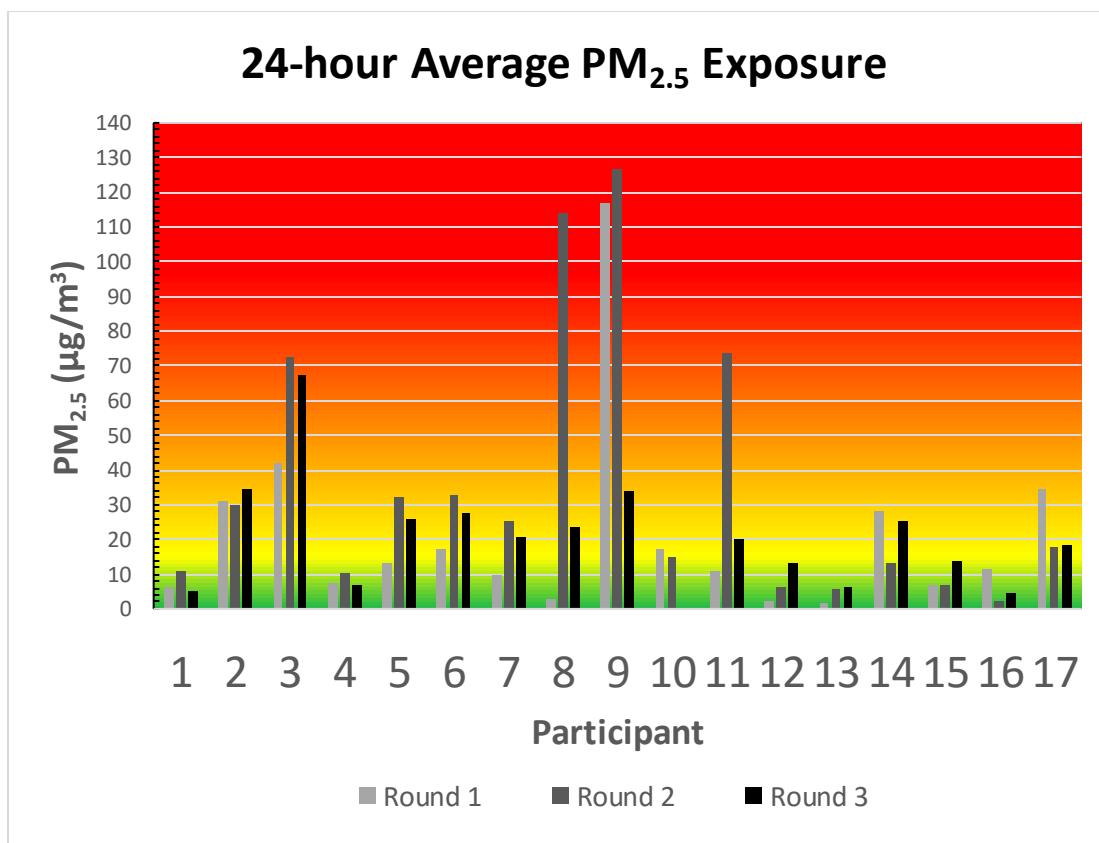
**Table 3.1 Summary Statistics of PM<sub>2.5</sub> Sampling Events**

PM <sub>2.5</sub> Summary Statistics				
N:	50			
Mean:	26.06 µg/m <sup>3</sup>		Lowest Value	1.92 µg/m <sup>3</sup>
Median:	17.22 µg/m <sup>3</sup>		Highest Value	126.74 µg/m <sup>3</sup>
Standard Deviation:	29.07		Range	124.82 µg/m <sup>3</sup>

When compared to the U.S. EPA Air Quality Index (EPA AQI) (Table 3.2), a majority of participants, over 82% (14/17), had at least one day at which their PM<sub>2.5</sub> exposure was above the EPA Air Quality Index Category “Good”. Furthermore, almost a quarter of participants (4/17) has at least one day at which their air pollution exposure was considered “Unhealthy” per the EPA AQI standards.

**Table 3.2 U.S. EPA Air Quality Index Categories**

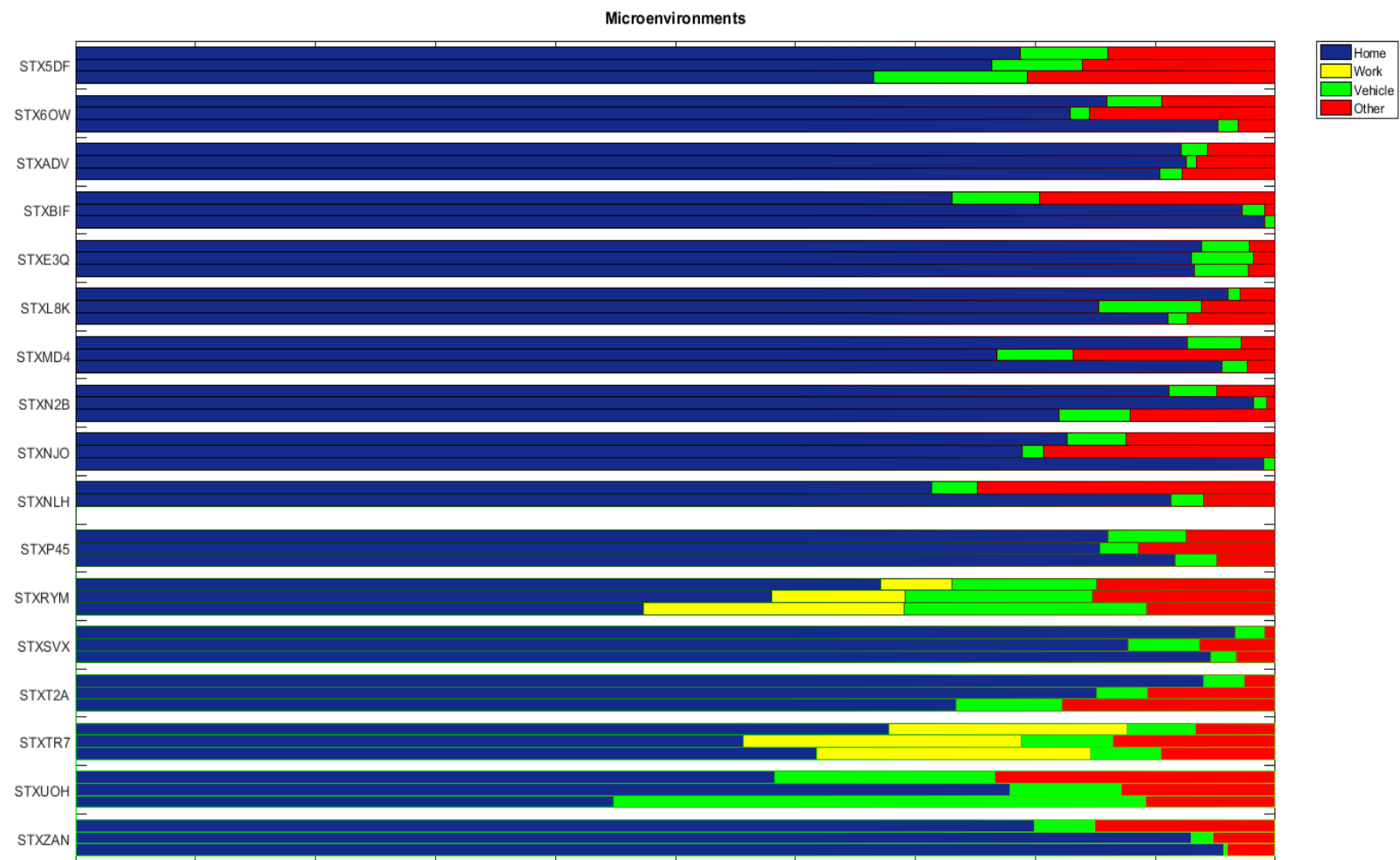
Air Quality Index Category	PM <sub>2.5</sub> 24 hour Average in $\mu\text{g}/\text{m}^3$
Good	0.0 – 12.0
Moderate	12.1 – 35.4
Unhealthy for Sensitive Groups	35.5 – 55.4
Unhealthy	55.5 – 150.4
Very Unhealthy	150.5 – 250.4
Hazardous	250.5+



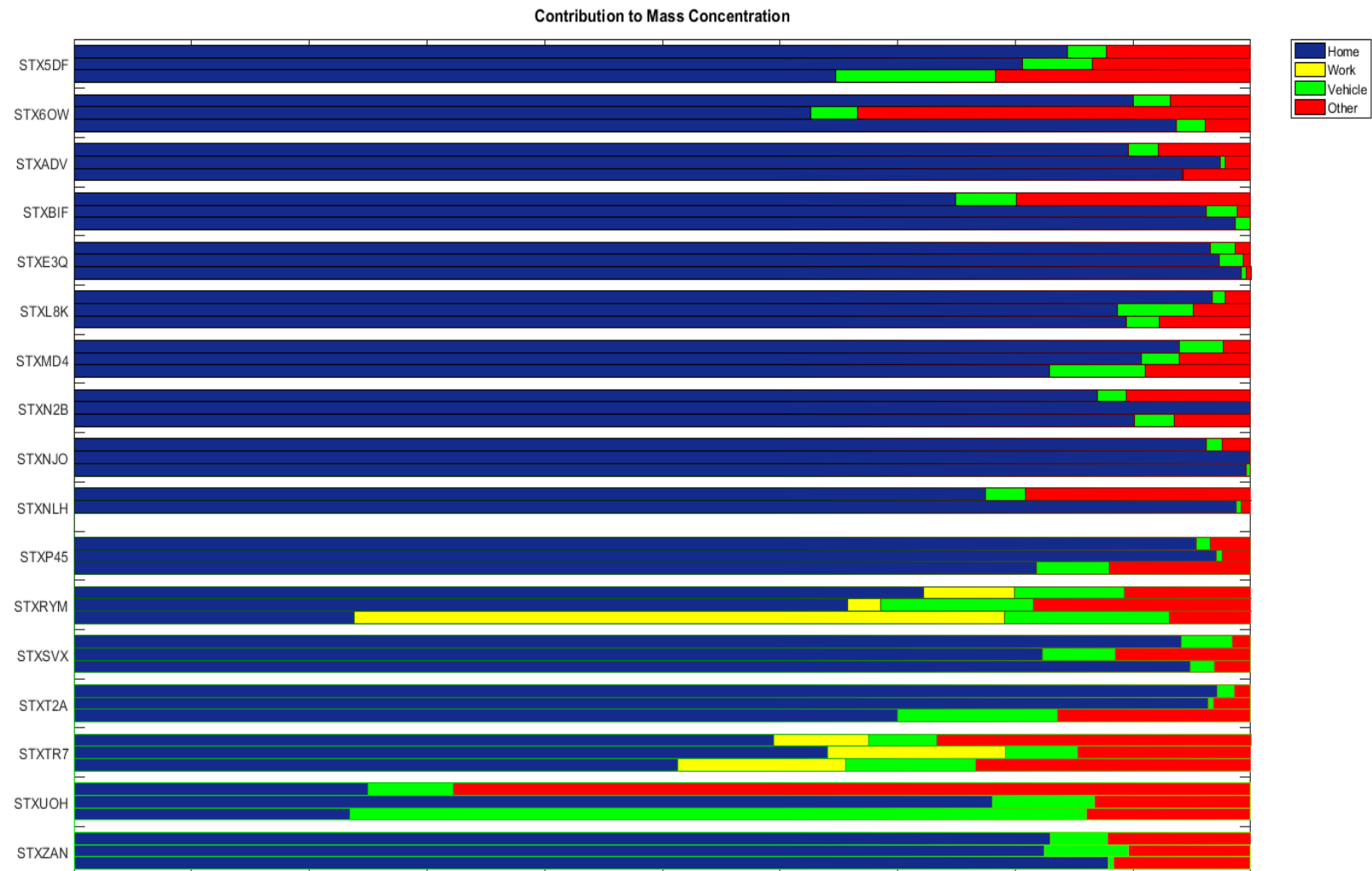
**Figure 3.1 24-hour average PM<sub>2.5</sub> exposure by sampling point.**

### **Section 3.2 Sources of PM<sub>2.5</sub>**

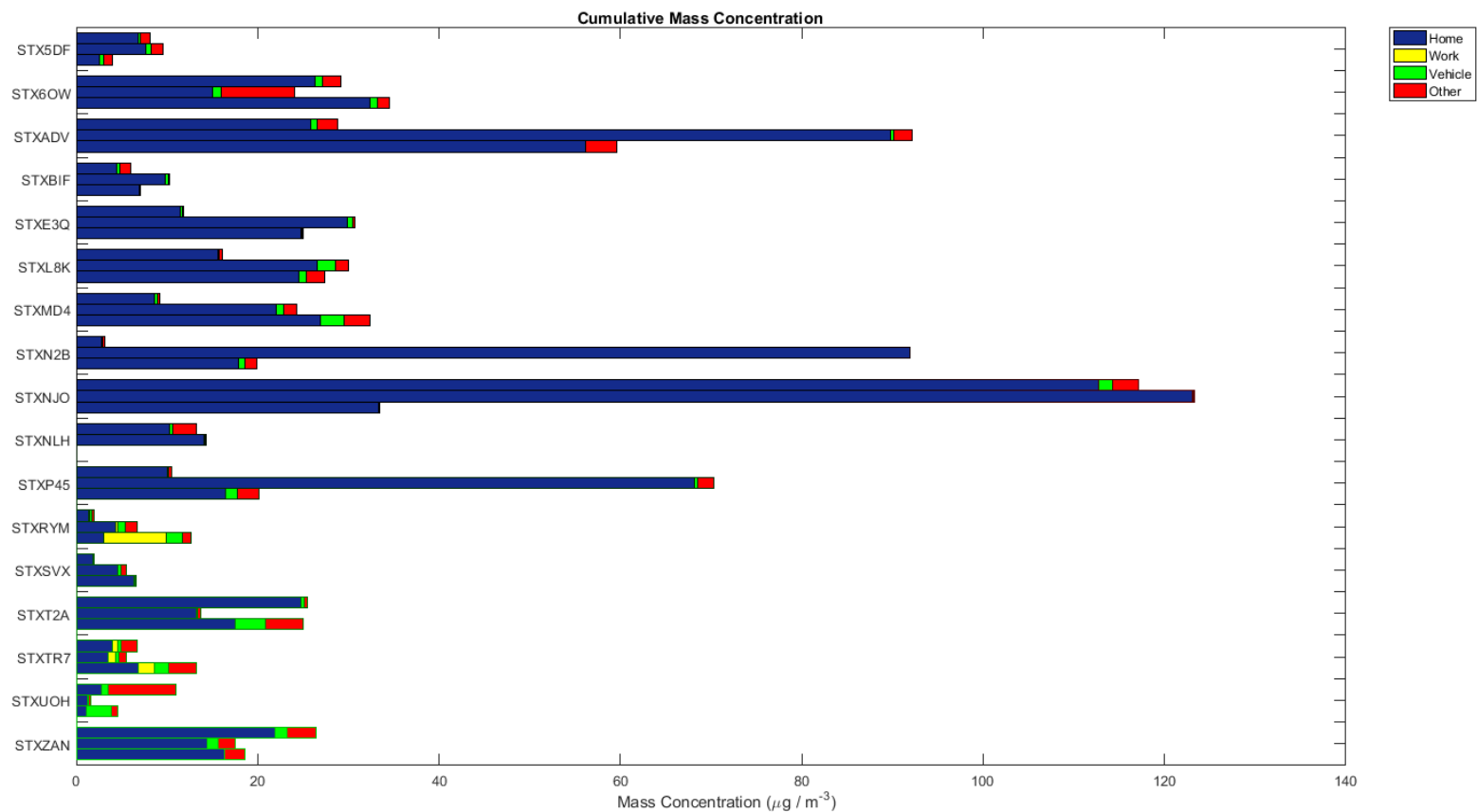
The sources of PM<sub>2.5</sub> were diverse and were categorized into the following microenvironments: home, work, time in a vehicle, and other, based on GPS data and time activity diaries. When stratified by microenvironments, the majority of sampled time occurred at home, however across sampling events, time at home ranged from 45% to almost 100% (Figure 3.2). These microenvironment patterns were generally consistent across each participant's sampling cluster (Figure 3.3). Work microenvironments were only identified in two participants and time in vehicle differed greatly among participants. When the contribution of these microenvironments to cumulative PM<sub>2.5</sub> mass was examined, PM<sub>2.5</sub> exposure from home was found to contribute overwhelmingly to cumulative PM<sub>2.5</sub> mass (Figure 3.4), with the exception of three sampling runs in which time in vehicle, work, and other, respectively, exposure contributed to the majority of cumulative PM<sub>2.5</sub> mass.



**Figure 3.2 Percent of sampling time spent in microenvironment.**



**Figure 3.3 Percent contribution of microenvironment to total PM<sub>2.5</sub> mass.**



**Figure 3.4 Cumulative mass concentration by microenvironment.**

### Section 3.3 Comparison to TCEQ Air Monitor

Participants active air monitor 24 average was compared to the Texas Commission on Environmental Quality (TCEQ) air monitor for the same intervals, the summary statistics of these sampling events are listed in Table 3.3. When compared via two-sided T-test with unequal variance no significant difference between the two sampling events was identified.

**Table 3.3 Summary Statistics of TCEQ PM<sub>2.5</sub> Sampling Events**

TCEQ PM <sub>2.5</sub> Summary Statistics				
N:	50			
Mean:	28.45 µg/m <sup>3</sup>		Lowest Value	1.93 µg/m <sup>3</sup>
Median:	17.83 µg/m <sup>3</sup>		Highest Value	164.15 µg/m <sup>3</sup>
Standard Deviation:	33.93		Range	162.23 µg/m <sup>3</sup>



#### 4. CONCLUSIONS

Air pollution exposure assessment is often a tradeoff between achieving coverage and identifying the intricacies of exposure. As air pollution exposure can vary greatly spatiotemporally, the role of one's microenvironment is a major factor in overall exposure, as TRAP levels have been shown to fluctuate even across relatively small distances (Ryan & LeMasters, 2007). Efforts to model air pollution exposure based on single 24 or 48 hour exposure windows may misclassify exposure due to variability between days, especially if the sampling period captures a rare contributor to air pollution, like an haze event or higher than normal time in a vehicle, or misses a regular contributor to air pollution that occurs outside of the sampling period. Furthermore, weather, seasonal variation, and alterations in personal behaviors can all contribute to the complexity of accurately assessing air pollution exposure. This study examined the utility of three, 24-hour time points, each roughly 2 weeks apart, to explore the role of the microenvironment on PM<sub>2.5</sub> exposure among pregnant women. Not surprisingly, the women sampled exhibited diverse exposures and unique microenvironments, which directly impacted their exposure. Home was the largest contributor to PM<sub>2.5</sub> mass which is understandable due to the large amount of time spent in the home microenvironment. In this population, however, it was postulated that traffic may be a major source of PM<sub>2.5</sub> due to the proximity of the study site to three major border crossings, which in total experienced a traffic volume of 546,259 heavy trucks, 25,776 buses, and 4,594,298

personal vehicles crossing into the U.S. in 2015 alone (US Department of Transportation, 2016). Despite the high volume of traffic nearby, the overall burden of PM came from the home environment, however, the source of the PM will have to be analyzed, comparing markers of TRAP to markers of general combustion before the amount of traffic derived particulate can be determined.

Also of note is the relationship between 24 hour PM<sub>2.5</sub> averages and the EPA Air Quality Index Standards. The majority of women (over 82%) experienced at least one day where their 24-hour sampling period was classified as less than good. This seemingly small increase in PM exposure is not without its risks as several studies have identified strong relationships between increases of PM<sub>2.5</sub> in 10 µg/m<sup>3</sup> increments and significantly increased risk of small for gestational age and low birth weight when exposed during pregnancy, as well as increased risk myocardial infarction, asthma symptoms, and overall mortality (McCormack et al., 2009; Shi et al., 2016; Stieb et al., 2016; WHO, 2013). Unfortunately, small for gestational age and low birth weight put children at increased risk of stillbirth and early mortality as well as obesity, metabolic disease, diabetes, and myocardial infarction later in life (Curhan et al., 1996; McCowan et al., 2010). As such, even seemingly minor increases in PM<sub>2.5</sub> should be viewed as an increase in risk and efforts should be made to reduce overall exposure, especially among pregnant woman and other susceptible groups. Roughly 23% of the women sampled reached levels deemed unhealthy by the EPA and in particular about 10% of the participants had consistently high PM exposures across their sampling cluster. These individuals represent

a population that warrants additional research and a potential intervention, as mentioned earlier their high exposure levels put them and their children at increased risk of negative health outcomes and thus even moderate reductions in PM exposure would reduce their risk of negative outcomes later in life.

Also of interest was the success of the TCEQ air monitor at measuring fairly consistent PM data in relation to the active air monitors. It was postulated that the TCEQ monitor would be less accurate than the active air monitor due to the air monitor's proximity to the participant. In some cases the TCEQ monitor over or under estimated participants exposure, but 68% of the sampling events the monitor was within 5% of the active air monitor values. This strong association seems to indicate that a significant amount of PM that the participants are exposed to is from the ambient environment, however, additional investigation is warranted to measure both the source of the PM and the reliability between the stationary monitor and the active air monitors.

The current state of air pollution exposure assessment is hampered by the variable nature of air pollutants and the impact of microenvironments on exposure. Additionally, although risk of health effects can be calculated based on overall PM<sub>2.5</sub> exposure, the measure is still limited in its generalizability. PM<sub>2.5</sub> originates from a variety of sources and as such equal exposure to PM from unique sources may elicit drastically different health effects and likely results in disparate exposure to PACs. To fully elucidate the health effects of PM exposure specific biomarkers of exposure is needed, as without which, the identification of those at highest risk is challenging and the discovery of

biological signatures of susceptibility is hindered, as an accurate internal dose cannot be reliably calculated (Morgott, 2014). Future work will investigate the presence of 1-nitropyrene, as well as the 16 EPA Priority PAHs to attempt to parse the contribution of traffic-related emissions to overall PM<sub>2.5</sub> in this region. This work will also examine the relationship between measured PACs in ambient atmosphere with urinary PAC metabolites in an attempt to assess the utility of 1-nitropyrene urinary metabolites as biomarkers of TRAP.

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